Supplementary Information

P-glycoprotein Inhibition Exacerbates Paclitaxel Neurotoxicity in Neurons and Cancer Patients

Stage et al. 2020

Figure S1. Eighteen day differentiation of SH-SY5Y cells generates cells with a complex neuronal network. Scale bar represents 200 µm.

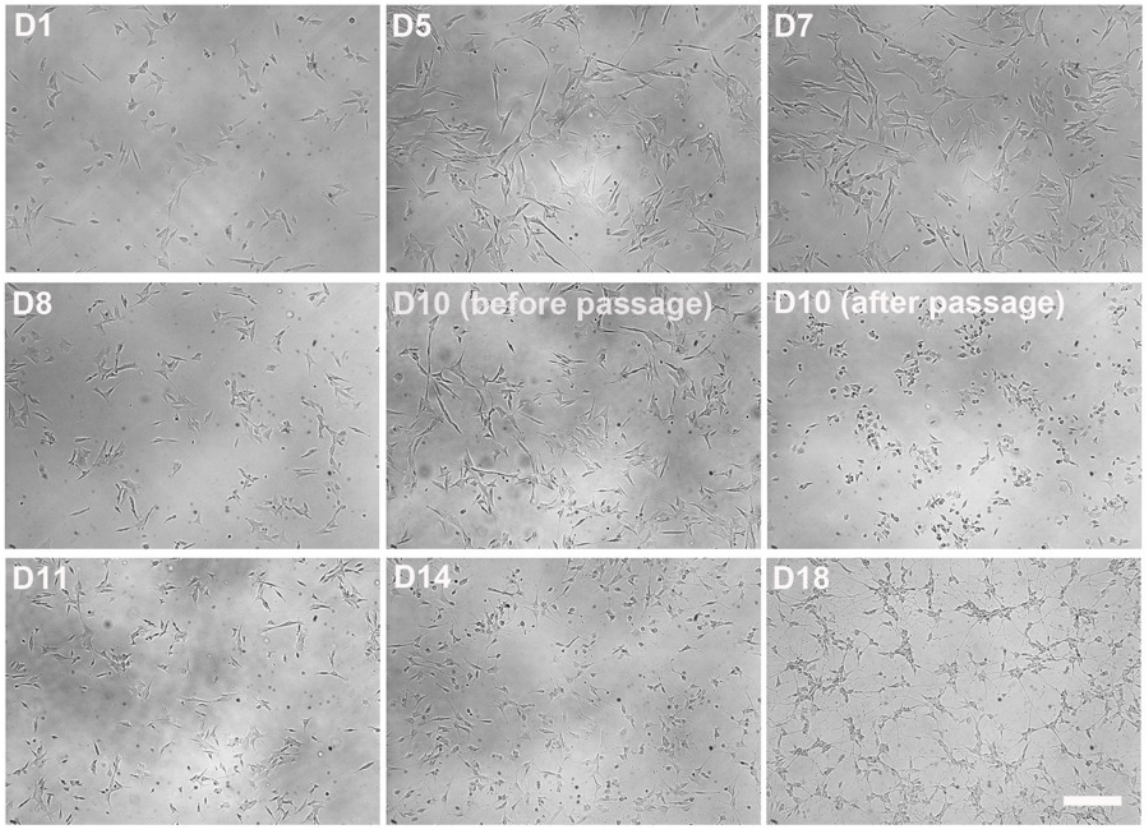
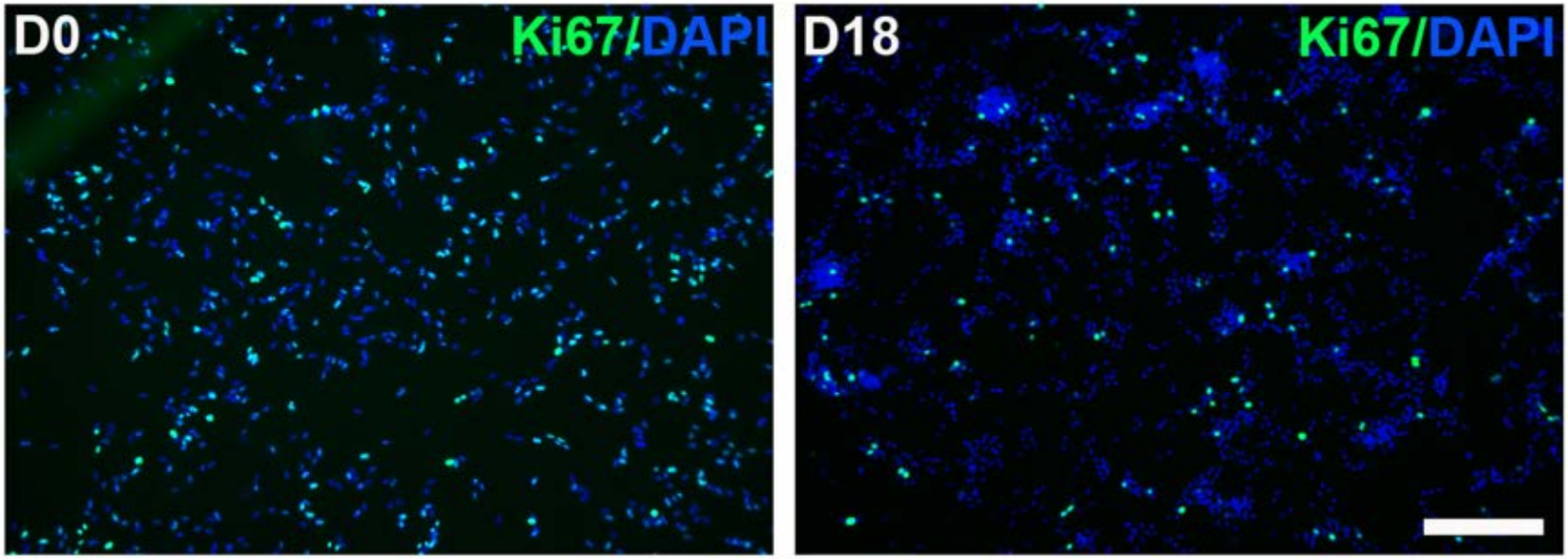
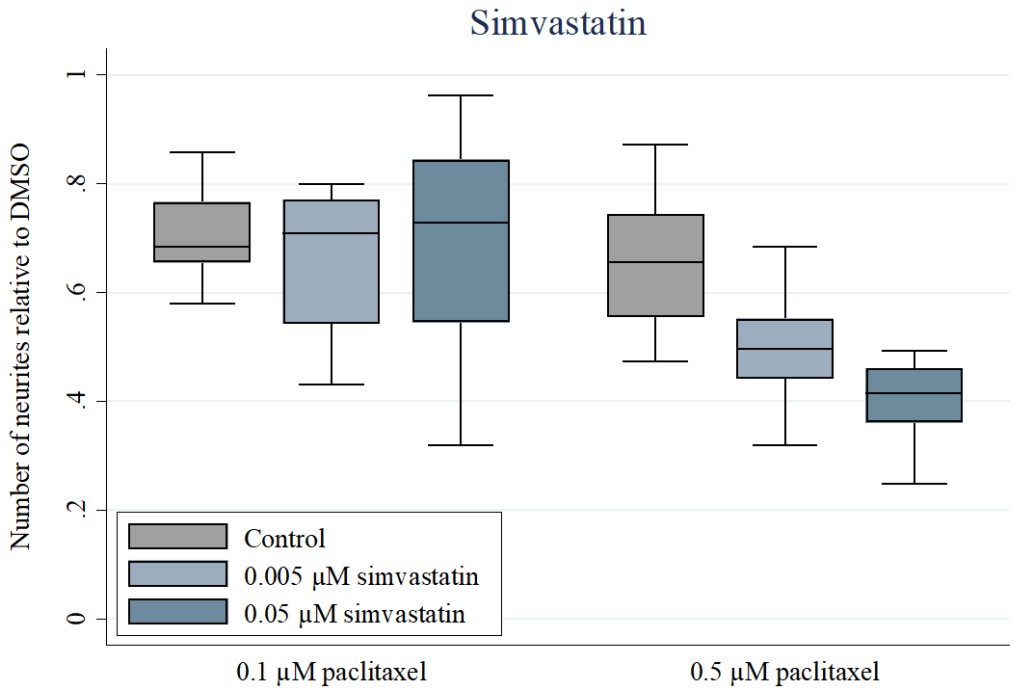


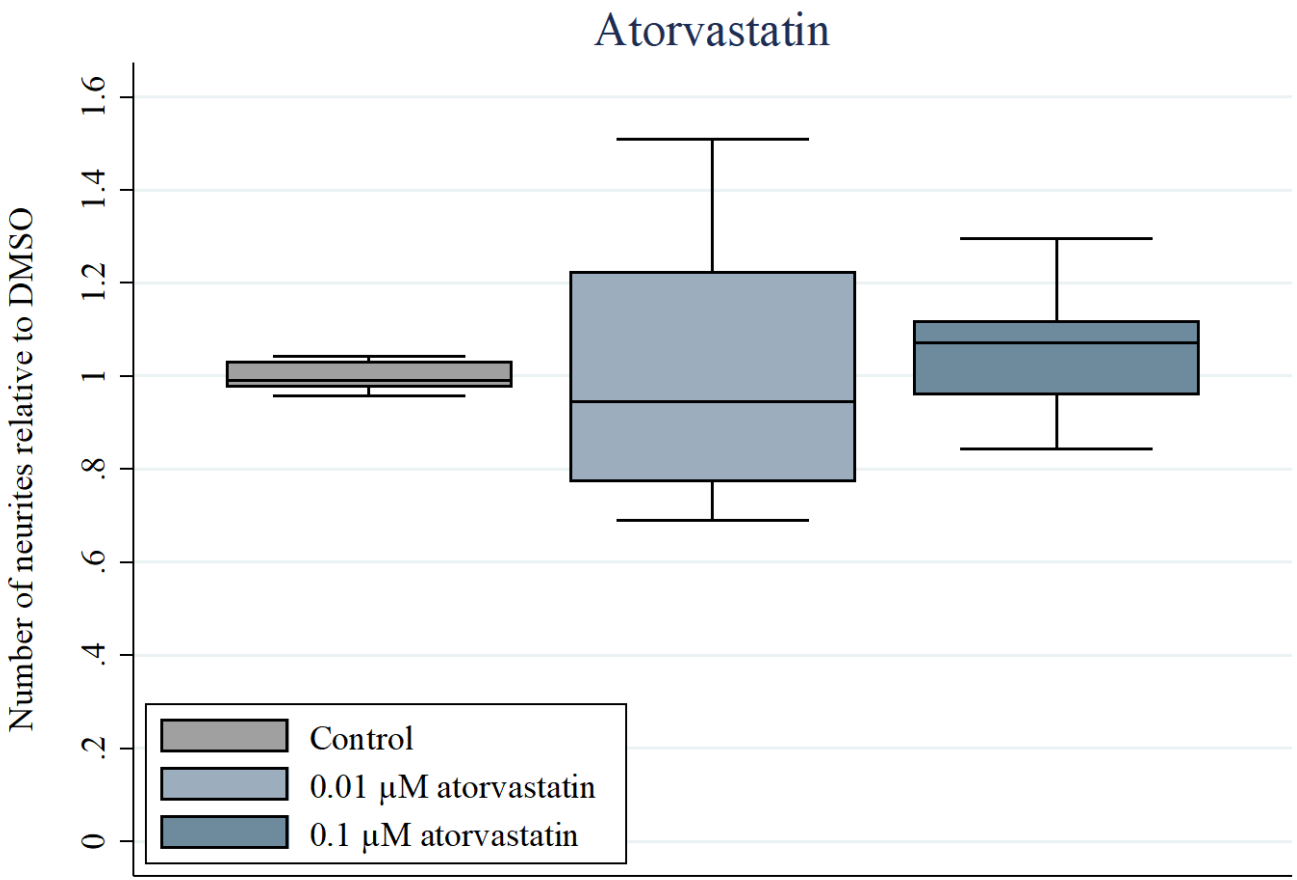
Figure S2. Immunolabelling of Ki67 before and after 18 days of differentiation of SH-SY5Y cells shows that a low proportion of cells proliferate, highlighting their post-mitotic state.



Supplementary Figure 3. Simvastatin exacerbated neurotoxicity only at a higher concentration of paclitaxel. Fully differentiated SH-SY5Y cells were treated with the indicated concentrations of paclitaxel for 24 hr in the absence and presence of simvastatin (0.005 or 0.05 μM) and stained for β-tubulin and nuclei. The number of neurites were quantified using ImageJ. At least five images from three separate differentiations were assessed. Simvastatin caused a significant exacerbation of neurotoxicity only at 0.5 μM paclitaxel ($p = 0.002$).



Supplementary Figure 4. Atorvastatin treatment alone did not cause neurotoxicity in SH-SY5Y cells. Fully differentiated SH-SY5Y cells were treated with the indicated concentrations of atorvastatin for 24 hr and stained for β-tubulin and nuclei. The number of neurites were quantified using ImageJ. At least five images from three separate differentiations were assessed.



Supplementary Figure 5. Simvastatin treatment alone did not cause neurotoxicity in SH-SY5Y cells. Fully differentiated SH-SY5Y cells were treated with the indicated concentrations of simvastatin for 24 hr and stained for β-tubulin and nuclie. The number of neurites were quantified using ImageJ. At least five images from three separate differentiations were assessed.

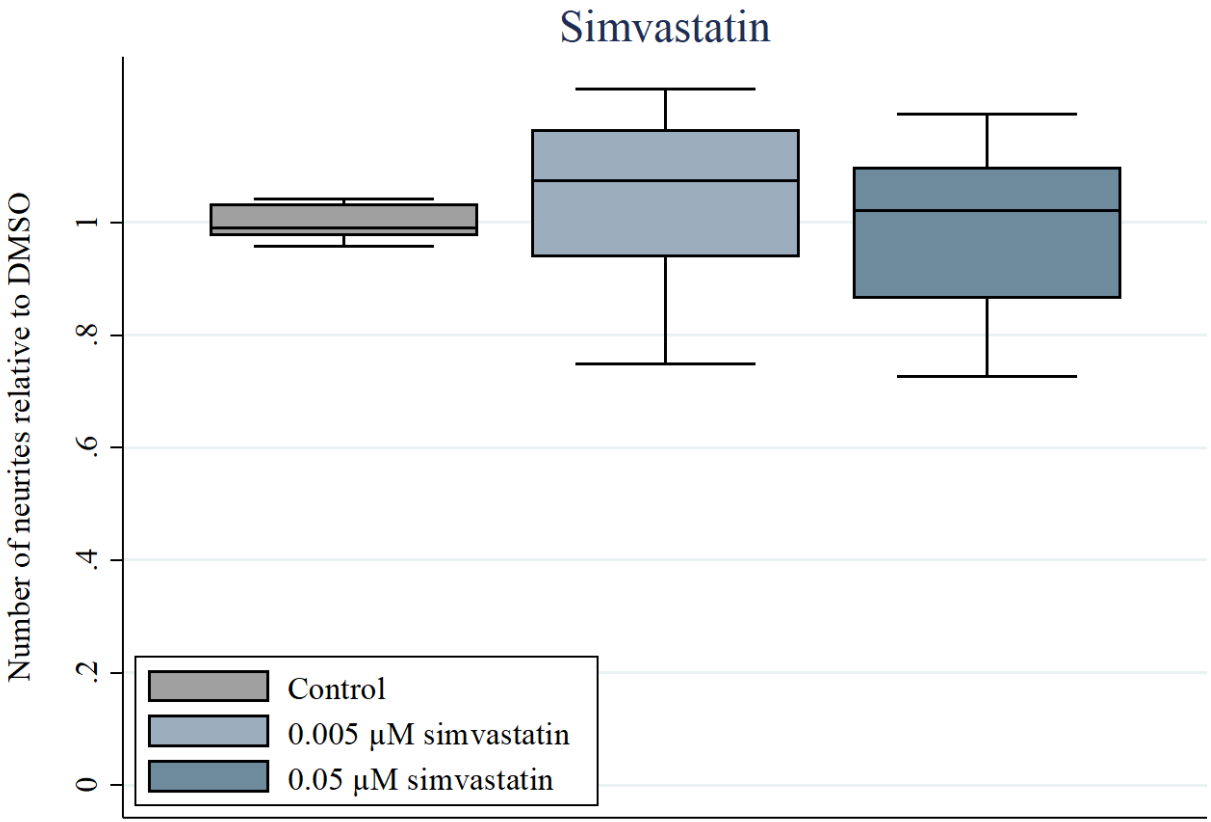


Table S1. Multivariate analyses of dose modification due to peripheral neuropathy.

Features	Any P-gp inhibitor		Strong P-gp inhibitor		Atorvastatin		Simvastatin	
	OR (95% CI)	**P**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**
P-gp inhibitor	2.36 (1.31, 4.25)	0.0043	4.73 (1.88, 11.9)	0.0010	7.01 (2.28, 21.5)	0.00067	0.33 (0.0, 1.19)	0.091
Tumor type	1.24 (0.39, 3.90)	0.71	1.11 (0.35, 3.47)	0.86	1.03 (0.33, 3.20)	0.96	1.26 (0.41, 3.86)	0.69
Stage	0.55 (0.28, 1.09)	0.084	0.52 (0.26, 1.06)	0.073	0.54 (0.27, 1.10)	0.092	0.63 (0.32, 1.23)	0.177
Age	1.03 (1.00, 1.06)	0.031	1.04 (1.01, 1.06)	0.013	1.03 (1.01, 1.06)	0.015	1.05 (1.02, 1.08)	0.0004
Paclitaxel, FEC/AC schedulue	0.21 (0.06, 0.71)	0.012	0.22 (0.06, 0.76)	0.017	0.23 (0.07, 0.79)	0.020	0.24 (0.07, 0.82)	0.022
Paclitaxel/carboplatin schedule	0.87 (0.29, 2.56)	0.80	0.97 (0.33, 2.85)	0.96	0.99 (0.34, 2.85)	0.98	0.89 (0.31, 2.59)	0.84
Previous chemotherapy	2.08 (0.34, 12.9)	0.43	1.64 (0.25, 10.8)	0.61	2.15 (0.35, 13.0)	0.41	1.81 (0.30, 11.1)	0.52
Body surface area	1.83 (0.60, 5.62)	0.29	1.80 (0.59, 5.49)	0.30	1.77 (0.58, 5.44)	0.32	1.89 (0.61, 5.82)	0.27

Table S2. Proteins analyzed by TXP targeted proteomic analysis

Protein	**UniProt ID**	**Peptide Sequence**
ABCB1 (P-gp)	P08183	NTTGALTTR
ABCC1 (MRP1)	P33527	ITIIPQDPVLFSGSLR
ABCC2 (MRP2)	Q92887	LTIIPQDPILFSGSLR

Table S3. List of P-gp inhibitors used to assess the impact of P-gp inhibition on the risk of dose modification of paclitaxel due to sensory neuropathy.

Group	**Lund et al. 2017 [31]**	**Wessler et al. 2013 [32]**
Anticancer and immunomodulatory	Carbozantinib	
	Ciclosporin A	Cyclosporine
	Darunavir	
	Enzalutamide	
	Everolimus	
	Ibrutinib	
	Imatinib	
	Lapatinib	
	Regorafenib	
	Tacrolimus	Tacrolimus
		Tariquidar
		Valspodar
Cardiovascular drugs	Amiodarone	Amiodarone
	Carvedilol	Carvedilol
	Diltiazem	Diltiazem
	Drondedarone	Dronedarone
	Ibresartan	
	Lomitapide	
	Propafenone	Propafenone
	Propranolol	Propranolol
	Quinidine	Quinidine
	Ranolazine	Ranolazine
	Simvastatin	
	Ticagrelor	Ticagrelor
	Verapamil	Verapamil
		Atorvastatin

		Captopril
		Felodipine
		Warfarin
		Dipyridamole
		Losartan
		Mibefradil
		Nicardipine
		Nifedipine
		Reserpine
		Talinolol
		Telmisartan
Infectious diseases	Azithromycin	Azithromycin
	Clarithromycin	Clarithromycin
	Daclastavir	Erythromycin
	Itraconazole	Itrazonazole
	Ivermectin	Ivermectin
	Ketoconazole	Ketoconazole
	Ledipasvir	
	Lopinavir	
	Mefloquine	Mefloquine
	Ponatinib	
	Roxithromycin	
	Saquinavir	
		Ofloxacin
		Rifampin (only single dose, multiple dose is an inducer)
CNS drugs	Flupenthixol	
	Fluvoxamine	
	Haloperidol	Haloperidol
	Paroxetine	
		Amitriptyline

		Chlorpromazine
		Desipramine
		Disulfiram
		Doxepin
		Fluphenazine
		Imipramine
		Sertraline
		Varenicline
GI drugs	Loperamide	
		Cimetidine
		Omeprazole
Hormones and drugs for endocrine disorders	Eliglustat	
Others	Mirabegron	
	Quinine	
		Conivaptan
		Elacridar
		Progesterone
		Troglitazone

Drugs that are highlighted with green are noted as strong/potent inhibitors in the respective references.